VIRUS REMOVAL DURING PHYSICOCHEMICAL TREATMENT OF RAW WASTEWATER

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CERTIFICATE

Certified that the work presented in this thesis entitled "Virus Removal During Physicochemical Treatment of Raw Wastewater" by Sri T.M. Prakash has been carried out under my supervision and it has not been submitted elsewhere for a degree.

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Available literature indicated that studies on virus removal efficiency of physicochemical treatment processes in wastewater treatment were very few and were unable to present a comprehensive picture. The objective of this investigation was to study the virus removal potential of various physicochemical processes, both separately and in combination, in the treatment of wastewater. Virus removal potential of plain sedimentation, alum and ferric chloride coagulation, and filtration through Giridih coal, activated carbon and Giridih coal-sand dual-media, from MS2 phage seeded wastewater, was evaluated.

Plain sedimentation of raw wastewater for 2 hr removed 21-28 percent of seeded virus. Both alum and ferric chloride were effective coagulants with identical maximum removal of 99.1 percent; however, the dosages were 240 and 340 mg/l, respectively. Giridih coal sorbed viruses better from alum coagulated and raw wastewater than sand. Activated carbon filter bed (71.12 cm deep) removed more viruses (59 percent) compared to Giridih coal bed (76.20 cm deep), which removed 36.2 percent of influent virus, when raw settled wastewater was filtered at a rate of 4.9 m/hr. Performance

of Giridih coal-sand dual-media filter when alum coagulated wastewater was filtered at rates of 4.9 m/hr and 9.8 m/hr was commendable. A consistent and good removal of viruses may be expected in a system consisting plain sedimentation, alum coagulation and filtration through Giridih coal-sand dual-media filter.

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1. INTRODUCTION

Recently there has been an increasing concern regarding the waterborne transmission of viral infections throughout the world. Many research workers have demonstrated the presence of over 100 sero types of viruses in human feces, which are subsequently isolated from raw domestic wastewater (Committee Report, 1970; and Engelbrecht, 1976). Enteric viruses have also been isolated from surface water samples collected throughout the world. Elmer et al. (1971), in their review, have illustrated the possibility of virus contamination of ground waters. In addition, there has been some evidence of transport of human enteric viruses from potential reservoirs in animal pets via storm water run-off. Numerous workers have studied the survival of enteric viruses in waters and have found them to survive for a significant length of time to consider water a potential route of viral disease transmission (Elmer et al., 1971).

The ever increasing demand of water throughout the world has led to the concept of 'reuse'. As a result of population growth and accompanying urbanization, the period between 'use and reuse' has been considerably narrowed down resulting in insufficient time available for significant natural inactivation or reduction of viruses and other pollutants. Therefore, there is a need for researching different methods of wastewater treatment for their maximum removal. Engelbrecht (1976) has rightly remarked "To date, field studies have provided relatively limited information

concerning the efficacy of wastewater and water treatment processes for removing viruses." Because of the lack of an acceptable technique to quantitatively detect a small number of viruses in large volumes of water, laboratory studies using large inocula have provided reasonable amount of information concerning the removal and/or inactivation of viruses by treatment processes. Extensive data on water treatment and conventional wastewater treatment processes are available. A summary of virus removal potential of these processes are given in Table 1 and Table 2. It is evident from Table 2 that the virus removal potential of conventional wastewater treatment processes vary widely and may not be reliable. Though addition of tertiary-level physicochemical processes subsequent to the conventional biological processes may considerably reduce the final effluent virus concentration, it incurs significant additional expenses. Further, the effective operation of a tertiary treatment system depends on consistent and efficient operation of the biological secondary process, which remains subject to problems arising from changes in waste composition, from large variation in flow which often have to be diverted, and from the presence of toxic materials which disrupt biological oxidation processes (Weber et al., 1970).

The concept of applying physicochemical treatment directly to a primary wastewater, rather than to a water which has undergone biological secondary treatment, is of recent origin. This represents a significantly different concept in the application of advanced processes for wastewater treatment. Weber et al.

TABLE 1

Removal of Viruses by Water Treatment Processes (Engelbrecht, 1976)

Processes	Removal or Inactivation (percent)
Softening	
Straight lime	10 - 70
Excess lime-soda ash	> 99.9
Coagulation and Flocculation	
Alum	98 - 99.9
Polyelectrolyte	36 - 99
Ferric chloride	> 99
Slow Sand Filtration (0.035 gpm/sq ft)	22 - 96
Rapid Sand Filtration (2-6 gpm/sq ft)	1 - 50
With coagulant and settling	90 - 99.7
Diatomaceous Earth Filtration (1 gpm/sq ft)	0 - 20
Coagulant coated (1 gpm/sq ft)	> 99
Chlorination	> 99.99

NOTE: Approximate virus input concentration:

$1 \times 10^4 - 1 \times 10^5$ PFU/ml.

TABLE 2

Removal of Viruses by Wastewater Treatment Processes (Engelbrecht, 1976)

Processes	Removal or Inactivation (percent)
Primary Treatment	
Grit chamber — comminutor	0 - 50
Plain sedimentation	0 - ?
Secondary Treatment	
Activated sludge	75 - 99
Trickling filter	0 - 85
Stabilization pond	0 - 96
Chemical coagulation — alum, iron salts	20 - 60
Advanced Treatment	
Chemical coagulation — alum, iron salts	90 - 99
Phosphate precipitation	90 - 98
Activated carbon adsorption	10 - 99

NOTE: Approximate virus input concentration:

$1 \times 10^4 - 1 \times 10^5$ PFU/ml.

(1970) demonstrated, by their pilot plant studies, the effectiveness and advantages of physicochemical processes over the conventional treatment processes. Very few studies on virus removal during physicochemical treatment of wastewater have been reported in the recent literature. These studies were not conducted in a systematic manner and do not give a comprehensive picture of the virus removal potential of physicochemical treatment processes in wastewater treatment.

The present work was undertaken to study in a systematic manner the virus removal during physicochemical treatment of raw wastewater. The treatment processes included were plain sedimentation, alum and ferric chloride coagulation, and filtration through coal, activated carbon and coal-sand dual media.

2. LITERATURE REVIEW

2.1 Viruses

2.1.1 The Nature of Viruses

Viruses serve as the border between the living and the inanimate. They are obligate intracellular parasites and outside a suitable host a virus becomes much like a colloid with no control over its position or capacity to multiply. Viruses are the smallest biological form capable of producing diseases in human and in other living species.

Basically, a virus is composed of genetic material contained by a coat. The genetic material is either single or double stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The coat or capsid enclosing the core of nucleic acid is usually formed by protein which protects the virus genome from unfavourable environmental conditions and promotes attachment of the virus. Because of the protein coat viruses exhibit an amphoteric behaviour when suspended in aqueous media and the net charge depends on the pH of the surrounding medium. Isoelectric point for most viruses being below 4.5, virus particles have a net negative charge at pH values of most natural waters and wastewaters.

Viruses have definite shapes and structures. The size of a virus may range from 10 to 300 nm. Most of the enteric viruses of concern are about 30 nm in diameter. Viruses may be spherical, cubical, helical, brick shaped or filamentous in shape. Viruses

of the same type are characterized by a definite size and shape.

Viruses can change the life process of a cell, causing injury, a modification in growth rate, or even death. Some viruses infect a cell without causing any recognizable effect. In such an instance, the virus can remain dormant indefinitely or can be stimulated to multiply.

Table 3 gives the families of viruses capable of infecting vertibrates. With the exception of the new family, Bunyaviridae, characteristics of each are represented in Table 4 which is based on a classification system proposed by Melnik.

2.1.2 Transmission of Viruses Through Water Route

Very little is known about the occurrence and behaviour of viruses in aqueous environments to represent a hazard. Mosley (1966) and Chang (1968) reported that any virus excreted in the feces may theoretically be transmitted through water. However, there are very few viruses for which epidemiological evidence suggests transmission by drinking water. The matter of waterborne potential becomes a function of the response of a virus to its aqueous environment, time involved before a suitable host is reached, succeptibility of the host, available portal for infection, initial concentration of infectious particles, etc. Boardman et al. (1977) suggested the following general rules which may identify the infectious agents more likely of significance

1. The virus would have to be emitted from the host in appreciable quantity.
2. The virus must be relatively stable in aqueous systems.

TABLE 3*

Virus Families of Vertebrates

Poxviridae	Papovaviridae
Parvoviridae	Retroviridae
Reoviridae	Paramyxoviridae
Rhabdoviridae	Orthomyxoviridae
Herpetoviridae	Togaviridae
Adenoviridae	Coronaviridae
Arenaviridae	Picornaviridae
Bunyaviridae	Unclassified Viruses

Unknown

*Adapted from Fenner (1976)

TABLE 4*

Properties of Vertebrate Virus Groups

Nucleic Acid Type	Number of Nucleic Acid Strands	Nucleocapsid Symmetry	Naked or Enveloped Virus	Virion Shape	Virion Size, nm	Family
DNA	1	Icosahedral	Naked	Spherical	18-24	Parvoviridae
	2	Icosahedral	Naked	Spherical	40-55	Papovaviridae
				Spherical	70-80	Adenoviridae
			Enveloped	Approximately Spherical	110	Herpetoviridae
		Complex	Naked	Brick Shaped	230x300	Poxviridae
RNA	2	Icosahedral	Naked	Spherical	54-75	Reoviridae
	1	Icosahedral	Naked	Spherical	18-30	Picornaviridae
			Enveloped	Spherical	35-40	Togaviridae
				Approximately Spherical	100	Retroviridae
		Helical	Enveloped	Approximately Spherical	80-120	Orthomyxovirid
				Pleomorphic	100x300	Paramyxovirida
				Bullet Shaped	60x225	Rhabdoviridae
		Unknown	Enveloped	Oval or Pleomorphic	110x130	Arenaviridae
				Approximately Spherical	80x160	Coronaviridae

*Adapted from Acton et al. (1976)

3. Aggregates of viruses have exhibited lower inactivation rates to natural and imposed agents. And so, the survival of viruses with the capacity to aggregate would be favoured.

Enteric viruses, which grow in or near the intestinal wall and are discharged in large numbers in feces, generally meet these criteria most closely. Table 5 lists potential waterborne viruses and the disease(s) with which they have been associated. Apart from the viruses listed in table 5, it should be noted that there is always the unknown element which may be represented by undiscovered viruses or the role of others not well understood such as the Retroviridae or Herpetoviridae (Boardman et al., 1977).

TABLE 5

Human Enteric Viruses (Engelbrecht, 1977)

Groups	Types No.	Core	Size (nm)	Associated Diseases
Enterovirus				
Poliovirus	3	RNA	28	Polio
Coxsackie				
Group A	26	RNA	28	Meningitis
Group B	6	RNA	28	Meningitis
Echovirus	29	RNA	28	Diarrhea, Respiratory
Adenovirus	30	DNA	60-80	Respiratory, Eye Infections
Reovirus	3	RNA	70	Diarrhea, Respiratory
Infectious Hepatitis	1(?)	?	15(?)	Infectious Hepatitis

Even though the above discussion may give a picture of the probable waterborne viruses, the true incidence of waterborne viral disease is difficult to estimate. Epidemiological techniques are limited in several aspects. Hence, investigators must prove beyond doubt that no other possible route exists and their procedures were sound (Boardman et al., 1977).

The doubt about poliomyelitis being waterborne still persists (Mosley, 1966). A vast majority of poliomyelitis outbreaks do not appear to satisfy the epidemiological criteria for waterborne transmission even though such outbreaks have been suspected from wastewater contaminated water supplies. Nevertheless, there still remains the possibility that under unusual circumstances, poliomyelitis could break out as a water-borne infection (Clarke and Chang, 1959).

It is generally accepted by epidemiologists that infectious hepatitis is the only disease caused by an agent, believed to be a virus, for which evidence of waterborne transmission exists (Mosley, 1966). However, it should be noted that the hepatitis agent(s) is yet to be isolated. Over 50 known epidemics of infectious hepatitis have been proven to be waterborne. Melnick (1971) discusses how the Delhi epidemic of 1955-56 was found to be waterborne by epidemiological data. Recently, gastroenteritis or diarrhea is believed to be transmitted through water supply. Boardman et al. (1977), through a collection of epidemiological data, emphasizes on viral gastroenteritis being waterborne.

The question of how many viruses are required to initiate an infection is very controversial. The nature of minimal infective dose (MID) data is dependent on the type of host system, condition of the host, history of the host, type of virus, whether the virus is attenuated or not, and how the virus is administered. Plotkin and Katz (1966) reported the data which are presented in Table 6. Seven of nine infants fed milk containing between 30 and 100 TCD_{50} of poliovirus type 3 (strain Fox) were infected. Two of three infants receiving Fox virus via a nasogastric tube were infected with 10 TCD_{50}. The authors reported that one cell culture infective dose was sufficient to infect man.

TABLE 6*

Infection of Infant Humans with Poliovirus Type 3 (Strain Fox)

Dose (TCD_{50})	Infection Rate
100-1000	4/4
30-100	7/9
10	2/3

*Adapted from Plotkin and Katz (1966)

Very little information concerning the response of hosts to hepatitis viruses is available. Ward et al. (1958) discovered that 100 mg of feces from an IH patient was infectious, while 1 mg was not.

Thus, the current MID concept is very controversial. A limited number of viruses have been investigated and the data available are minimal because of the various limitations.

2.1.3 Detections of Viruses in Natural Waters and Their Densities

It is now obvious that the potential health problem posed by waterborne virus is not clearly defined. The lack of reliable, quantitative techniques have seriously limited the detection of viruses in the water environment and the evaluation of treatment methods in removing viruses from water and wastewater. Methods currently utilized to evaluate the potential for viral disease transmission may be classified as either direct or indirect. In the direct method an attempt is made to isolate the viruses, whereas in the indirect method, organisms possibly indicating the presence of viruses are examined.

Direct Methods:

The problems associated with looking for viruses directly are generally related to one or more of the following (Boardman et al., 1977)

1. small size
2. low concentration
3. instability
4. quality of water
5. assay procedures.

As previously indicated, viruses of significance have a size of 15-30 nm. Hence they are not visible through regular light microscopy.

The density of viruses in natural, aqueous systems is usually rather low. However, ~~qualities~~ recoverable may vary considerably from one month to another, from one city to another, from one country to another, etc. Considerable research has been carried out in estimating virus levels in wastewater. Kelly and Sanderson (1960) reported that the density of enteroviruses in domestic wastewater varied from less than 200 to 4000 PlaqueForming Units (PFU) per liter. Peak levels were normally found in late summer and early fall. Lund et al. (1969) found the density of viruses to vary with the season of the year in the temperate zone. They reported the maximum density of the order of 10^5 virus units per liter. According to Gerba et al. (1975a), enteric virus levels greater than 400,000 infectious particles per liter may be reached in raw domestic wastewater. It is difficult to interpret or relate these various findings because of the lack of uniformity in the method of sample concentration, the host cell system, and the type of culture techniques used (Engelbrecht, 1976).

Instability of viruses may influence detection methods. Viruses may remain viable long enough to initiate infection, they may not persist through the period of time required to process a sample. Also sampling and assay procedure may cause stress. The qualities of water like presence of turbidity and organisms which can degrade viral protein etc. may interfere with the means utilized for virus concentration and detection. Hence, it is important to consider biological, chemical and physical characteristics of the sample (Boardman et al., 1977). Due to the

idiosyncratic nature of viruses, no universal procedure or system is presently available for the cultivation of all viruses. Hence, there may be problem of assaying for viruses due to the error involved in certain procedures such as making dilutions, the tedium involved in routine operation, selectivity of the techniques employed etc.

The normal sequence of procedures utilized to isolate viruses by the direct method is concentration of viruses from a representative sample followed by assay and sometimes identification of the viruses. Low densities of viruses in natural waters require that large quantities of water be collected and subsequently concentrated before applying conventional virological detection and isolation techniques. In some cases a grab sample or direct inoculation method may be used. Obviously the relative success of this method is a function of virus concentration. However, Buras (1974) reported good recovery of enteroviruses from sewage and effluent samples through a direct incoulation method. Berg (1966) introduced the gauze pad method, which is somewhere between direct inoculation and concentration techniques. In this method, pads of gauze or sanitary napkins are suspended in flowing water for several days. Hence some degree of concentration may occur, but the method is by no means quantitative.

The following are the recently reported concentration methods:

1. Adsorption to filters (Jakukowaski et al., 1975; and Hill W.F. et al., 1976).

2. Chemical Precipitation (Rao et al., 1968).
3. Association with various materials (Moore et al., 1974).
4. Phase partition (Albertson, 1974).
5. Reverse osmosis (Sweet et al., 1971).
6. Hydro extraction (Moore et al., 1974).
7. Ultrafiltration (Sweet et al., 1971).
8. Ultra centrifugation (Cliver and Yeatman, 1965).
9. Electrophoresis (Bier et al., 1967).
10. Electro-osmosis (Hill et al., 1971).
11. Freeze concentration (Rubenstein et al., 1971).
12. Affinity chromatography (Grabow and Pozesky, 1973).

Boardman et al. (1977) have extensively reviewed the principle of operation and discussed the relative advantages of the above concentration methods. The membrane filter procedure of Cliver (1966) has been tentatively recommended for quantitative studies (Standard Methods, 1971). Recently, attempts have been made to develop portable virus concentrator for testing water in the field (Wallis et al., 1972).

Indirect Methods

Many attempts have been made for indirectly assessing viruses through measuring levels of coliform indicators. However, there has been serious objection regarding the validity of this practice (Goldreich and Clarke, 1971). Studies have been conducted to correlate the presence of yeast, acid-fast bacilli, bacteriophages

and algae with virus density by many research workers (Engelbrecht et al., 1974; and Dhillon et al., 1976). But so far no reliable indirect method has been standardised.

2.2 Survival of Viruses in Soil and Aquatic Environment

Following a review of the literature in this area, Gerba et al. (1975) concluded that the removal of viruses by soil is principally due to adsorption. Salt concentration, pH, presence of organic matter, soil composition and flow rates may all affect the degree of retention of viruses by soil particles. Generally, greater virus removals are accomplished at lower flow rates, pH levels, and concentrations of soluble organics, while low levels of cations and clay tend to decrease virus removal.

Regarding the laboratory studies of virus movement through soil, Drewry and Eliassen (1968) showed that nine different soils from California and Arkanas were capable of removing over 99 percent of the viruses (T1, T2, and f2 bacteriophages). Later, Drewry (1969) observed 99 percent virus adsorption on three of the four soils studied using f2 bacteriophages. Young and Burbank (1973) observed that only 35 percent of virus was removed or retained by 15 in. column of Tantatus soil which was characterised by rapid drainage. They also observed that even 6 in. columns were unable to effect 100 percent retention of poliovirus with initial feed concentration of 1.5×10^6 PFU/ml in a 2.5 in. soil column. Koya and Chaudhuri (1977) found three Indian soils, viz., Lateritic soil, Black cotton soil and Kanpur silt were effective in removing

viruses from water in terms of both batch sorption tests as well as the column studies. Virus particles retained in the soil were not inactivated and virus retention was dependent on the type and amount of the clay content as well the chemistry of system.

Major field tests concerning the movement of viruses in groundwater were conducted by Merrell and Ward (1968) at the Santee Water Reclamation Project at Santee, California. They concluded that the virus was removed in less than 200 ft of travel. Recently, Wellings et al. (1974) isolated viruses in wells 10 ft and 20 ft below the soil surface in a wastewater reclamation pilot project near St. Petersburg, Florida. No virus was detected in these wells for the first 5 months of the study. Hence, the retention of viruses by soil particles does not result in their permanent immobilization from the liquid phase, and changes in water quality can result in their deadsorption and further subsurface travel.

Considerable data are available concerning the viral contamination of surface waters. From an extensive review of existing information on the occurrence of viruses in ground and surface waters, Akin et al. (1971) concluded that enteric viruses were detected in 36 percent of the samples of surface water examined. It was also observed that viruses were isolated from surface waters throughout the world. From the review, it was concluded that the survival of enteric viruses in various waters was a function of temperature, mineral and organic matter in water, nature of water (lake, stream, well), rate of water flow, etc.

Many research workers have studied the persistence of viruses in different waters. Joyce and Weiser (1967) reported that Poliovirus type 1, Coxsackievirus, and Echovirus generally survived longer than 60 days in farm pond waters. Kott (1975) from their experiments found, at lower temperature, over 231 day survival of Poliovirus in tap water and distilled water.

Although not defined very well at this time, there is some feeling that algae, aggregation, and adsorption may influence virus survival. Several investigators have indicated that viruses associate readily with various materials in a suitable environment (Carlson et al., 1968; and Moore et al., 1975). But, little is available to correlate adsorption level with inactivation rate in fresh water systems.

2.3 Virus Inactivation or Removal During Water and Wastewater Treatment Processes

The following section considers the response of viruses to various water and wastewater processing operations. Although a tremendous amount of information is available, much of the data is intrinsically weak because the work was performed in the laboratory with either attenuated strains or phage. For these reasons, probably some degree of caution should be exercised in generalizing the available information with respect to the behaviour of indigenous enteric viruses. It may also be mentioned here that most of the available data on virus removal by the physicochemical treatment processes were obtained in terms of their application in water

treatment; however, the available information is presented here to provide a background for the present study.

2.3.1 Primary Sedimentation

The available evidence indicates that little or no removal of virus may be expected during primary sedimentation. However, rather high removals have sometimes been reported. The discrepancy appears to be related to the nature of the virus-solids association, character of the waste, and agitation in the system (Boardman et al., 1977). Agitation may sheer viruses from settleable matter or place viruses in a position where they may be eluted. Sherman et al. (1975) reported removals of seeded f2 phage ranging approximately 28 to 54 percent and 13 to 69 percent in the primary basins of two trickling filter plants. Clarke et al. (1961) found that levels of Coxsackievirus A9 and Poliovirus type 1 (Strain Mohoney) were not significantly reduced after 3 hours of settling. Other studies have indicated limited or inconsistent and undependable removal of viruses by primary sedimentation (Mack et al., 1962; Bush et al., 1966; and England et al., 1967).

2.3.2 Coagulation

Several investigators have indicated that coagulation-flocculation processes effectively remove viruses. The data presented in Table 7 illustrate that removals of viruses greater than 90 percent are readily attained. It is important to note that viruses may generally be recovered quite readily from the floc

TABLE 7

Removal of Viruses by the Coagulation - Flocculation Process

Investigators	Coagulant	pH	Virus(es)	Virus Removal (percent)	Turbidity	Turbidity Removal (percent)
Chang et al. (1958)a	40 mg/l $Al_2(SO_4)_3$	6.2	coxsackie A2	86.3	$S:O_2$	--
			phage	93.5	$S:O_2$	--
	40 mg/l $FeCl_3$	6.2	coxsackie A2	98.1	$S:O_2$	--
			phage	99.9	$S:O_2$	--
Chang et al. (1958)b	25 mg/l $Al_2(SO_4)_3$	6.7-7.4	coxsackie A2	99.0	<226 ppm (Ohio River Water)	
	15 mg/l $Al_2(SO_4)_3$	6.7-7.4	coxsackie A2	95.7	-ditto-	--
Foliguet and Doncoeur (1965)	60 mg/l $FeCl_3$	5-8	polio 1	99.9 (including filtration)	25-200 mg/l Montmorillonite	>97.6 (including filtration)
Manwaring et al. (1971)	80 mg/l $FeCl_3$	6.1	MS2 phage	99.5	--	97.5
Chaudhuri and Englebrecht (1970)	50 mg/l $Al_2(SO_4)_3$	5.1-5.4	T4 phage	95.71	18 JTU (settled waste-water added)	98.63
	50 mg/l $Al_2(SO_4)_3$	5.1-5.4	MS2 phage	89.60	19 JTU (settled waste-water added)	92.10
Shelton and Drewry (1973)	15 mg/l $Al_2(SO_4)_3$	6.8	f2 phage	99.45	1.2 JTU (unpolluted surface water)	96.50
	40 mg/l $FeCl_2$	6.8	f2 phage	99.10	1.2 JTU (unpolluted surface water)	94.80

Table 7 (continued)

Investigators	Coagulant	pH	Virus(es)	Virus Removal (percent)	Turbidity	Turbidity Removal (percent)
Shelton and Drewry (1973)	76 mg/l $Al_2(SO_4)_3$	7.1	f2 phage	99.60	3.9 JTU (domestic wastewater)	93.60
	109 mg/l $FeCl_3$	6.8	f2 phage	94.60	3.9 JTU (domestic wastewater)	90.00
York and Drewry (1970)	25 mg/l $Al_2(SO_4)_3$	8.1	f2 phage	99.9	14 JTU (lake water)	96.0
	50 mg/l $FeCl_3$	8.1	f2 phage	99.4	14 JTU (lake water)	92.5
	50 mg/l $Fe_2(SO_4)_3.H_2O$	8.1	f2 phage	92.0	14 JTU (lake water)	89.0
Wolf et al. (1974)	103 mg/l hydrated alum (Al:P ratio 0.44:1)	6.5-7.4	polio 1	63.0	8.5-12.0 FTU (secondary effluent)	~82
	-ditto-	6.5-7.4	f2 phage	46.0	8.5-12.0 FTU (secondary effluent)	~82
Parkhurst (1977)	150 mg/l alum and 0.2 mg/l anionic polymer	7.3	polio 1	95.0	4.6 FTU (secondary effluent)	--

Adapted from Boardman et al. (1977)

materials and organic matter may interfere with the effectiveness of this process to remove viruses.

Cationic polyelectrolytes were found effective in removing viruses from water both as prime coagulants and coagulant aids (Chaudhuri and Engelbrecht, 1970; and Throup et al., 1970). It was also demonstrated that the concentration of cations in the water affected quite markedly the extent of virus removal when polyelectrolytes were used. Shelton and Drewry (1973) indicated that anionic, nonionic and cationic polyelectrolytes did not significantly influence removals of f2 phage. The performance of polymers may be a function of polymer type and concentration, interference caused by substances in the medium, and virus type (Boardman et al., 1977). In general, good floc formation achieves virus removals which are not significantly improved by the addition of polyelectrolytes.

2.3.3 Filtration

Data for the removal of viruses by filtration systems are presented in Table 8. A review of the literature cited in Table 8 will lead to the following conclusions:

1. high, but not complete, removal of viruses in filtration systems is achieved utilizing a coagulant or polymer,
2. the performance of the filter diminishes with time, is inhibited by organic materials, and increases with decrease in flow rate, and

TABLE 8

Removal of Virus by Filtration Systems

Investigators	System	Virus	Observations
Carlson et al. (1942)	30 inch column of sand	polio adapted for mice	little virus removed; significant increase in removals when surface layers impregnated with alum
Robeck et al. (1962)	24 inch column of sand	polio 1 (Mahoney)	< 20 percent removal at 1 to 4 gpm/sq ft; addition of alum ahead of filters increased removals to 90-99 percent
Safferman and Morris (1976)	mixed media pilot plant filter	f2 phage	0-48 percent removals
Berg et al. (1968)	8 inch sand bed	polio 1 (LSc)	About 98.6 to 99.995 percent removal when lime added ahead of filter
Brown et al. (1974)	diatomaceous earth filter	T2 phage	>99 percent removals using filter aids
Chaudhuri et al. (1977)	17.6 inches of sand	MS2 phage	30 to 70 percent removal when viruses were seeded into ground water; 90 percent removal following alum cogulation of ground water containing kaolinite turbidity

Table 8 (continued)

Investigators	System	Virus	Observations
Amirhor and Engelbrecht (1975); and Chaudhuri *et al.* (1974)	diatomaceous earth filter	MS2 phage T4 phage	little removal from water with uncoated media; good removals from water using polymers to coat media or prior to filtration
Brown *et al.* (1974)	diatomaceous earth filter	T2 phage polio	initial removals >99 percent but slowly diminishing
Sorber *et al.* (1972)	ultrafiltration-reverse osmosis system	T2 phage polio	99.2 to >99.999 percent rejection of T2 and polio
Sriramulu and Chaudhuri (1976)	9 inch Bituminous coal over 9 inch sand	MS2 phage	92 percent removal following alum coagulation of ground water containing kaolinite turbidity
Parkhurst (1977)	24 inch anthracite coal over 18 inch sand	polio 1 (chat)	91 percent removal following anionic polymer aided alum coagulation of secondary effluent

Adapted from Boardman *et al.* (1977).

3. higher levels of virus in backwash water than in the raw water due to virus concentrating effects in coagulation and filtration operations.

Removal of viruses in absence of turbidity particles, is usually poor with rapid rate filtration (Sriramulu, 1975). However, the efficiency of virus removal substantially increases with turbid waters and when polyelectrolytes or coagulants are introduced with virus or allowed to impregnate the filter media. The performance of diatomaceous earth filters is also improved by such filtration aids.

2.3.4 Softening and Lime Treatment

The data given in Table 9 have been taken from a review by Sproul (1971). It can be clearly seen from this data that greatest virus removals of Poliovirus 1 was achieved by excess lime-soda ash softening of calcium and magnesium hardness. It may be generalised that virus removed by softening process may be a reflection of inactivation due to elevated pH levels and association with precipitating matter subsequently removed by sedimentation (Berg et al., 1968; and Thayer and Sproul, 1966).

Berg et al. (1968) observed 99-99.9 percent removal of Poliovirus 1 seeded into primary effluent by precipitation with either 400 or 500 mg/l of lime. Thayer and Sproul (1966) reported 90 percent inactivation of T2 phage after 90 min of contact when sufficient lime was added to distilled water to produce a pH level of 10.5. It is observed from the above studies that enteric

TABLE 9

Removal of Poliovirus 1 by Water Softening Operations (Sproul, 1971)

Softening Process	Initial Conditions (mg/l as $CaCO_3$) Hardness $MgCl_2$	Hardness $Ca(HCO_3)_2$	Alkalinity	Final Conditions Hardness Removal (mg/l as $CaCO_3$) Mg	Ca	pH	Poliovirus Removal (percent)
Straight Lime	--	100	106	--	64	9.0	9
	--	300	306	--	218	8.1	70
Precipitation of Mg with NaOH	100	0	nil	56	--	10.3	99.26
	300	0	nil	277	--	10.4	99.88
Excess Lime-Soda Ash	67	133	100	50	76	10.8	99.90
	100	300	200	82	204	11.2	99.993

viruses are relatively stable at pH levels reaching 10.5 to 10.8, but generally lose infectivity rapidly above pH 11.0.

2.3.5 Biological Processes

Activated Sludge

A number of studies have indicated that the activated sludge process is capable of removing viruses to the extent of 90 percent or more (Committee Report, 1970). Clarke et al. (1961) reported that a laboratory scale, continuous flow activated sludge unit accomplished approximately 90 and 98 percent removals of Poliovirus type 1 and Coxsackievirus A9, respectively in 6 to 7 hr of retention. The authors concluded that the adsorption onto the suspended and colloidal materials is a prime factor in virus removal in the activated sludge process. Kelly et al. (1961) also reported that there were certain bacteria in the activated sludge which displayed antagonistic activity to viruses.

Lund et al. (1969), in a field study, estimated that 95 to 99 percent of viruses were removed by an activated sludge system on the basis of the frequency of isolations in the influent and effluent. The removal of bacterial virus f2 was studied by Safferman and Morris (1976) utilizing a three-stage activated sludge pilot plant (0.05 mgd) consisting of modified aeration, nitrification and denitrification with alum addition, and filtration. The total treatment system achieved 99.97 percent removal of bacterial virus. Rao (1976) reported 99 percent reduction of indigenous enteric viruses by a pilot oxidation ditch having a detention period of 9.7 to 88 hours and MLVSS of 1000 to 9500 mg/l.

Trickling Filters

Trickling filters generally behave erratically and have rather low virus removal efficiency (Berg, 1973). Sherman et al. (1975) reported the removal of seeded f2 phage in two Maryland trickling filters as 6 to 40 percent in one plant and 1 to 13 percent in the other. These percentage removals did not include the effect of final classification. The average virus removal increased to 37.4 and 49 percent for the two plants, respectively when the effluent from the final classifier was considered. However, Clarke and Chang (1975) reported efficiencies as high as 95 percent in their bench scale, rotary tube trickling filters, at filtration rate equivalent to 10 mgd/acre. They could not recover viruses from biological layers on the filters. This indicated that either the virus-slime association was stable or the viruses were indeed inactivated.

Stabilization Ponds

A review of the published data indicates stabilization ponds are quite effective in reducing virus levels of raw wastewater and treated effluents. Biological and chemical constituents, detention time, adsorption to settleable solids, temperature and sunlight all may play significant roles in the removal or inactivation of viruses in oxidation ponds (Chaudhuri, 1973). The review by Chaudhuri (1973) has shown the removal of viruses by ponds to be erratic. Merrel and Katko (1966) reported 16 percent positive results for viruses in 63 effluent samples tested from oxidation ponds at

Santee, California. Arceivala et al. (1971) reported more than 90 percent removal and Nupen (1970) could not recover viruses from the effluent when the influent contained 240 PFU/l.

2.3.6 Adsorption

Cookson (1967, 1969 and 1970) has published most of the work on the removal of viruses from sewage effluents by filtration through activated carbon. He reported only 35 percent removal of T4 phage at a filtration of 19 litres/min/m^2. The virus removal was diffusion limited and reversible. Oliver (1971) investigated the use of activated carbon columns for the removal of Poliovirus type 1 (Strain CHAT), Coxsackievirus B3 (Strain Nancy), and Coxsackievirus A9 (Strain P.B.) from tap water, river water, or wastewater. Viruses associated with the carbon to some degree, but not to an extent that would insure safe effluents. Pilot plant studies at Pomona Valley, Los Angeles County reported 82 to 99.5 percent removal of the seeded virus by the gravity carbon filtration of secondary effluent (Parkhurst, 1977). Sproul et al. (1967) studied activated carbon adsorption of Type 1 Poliovirus from a secondary effluent, which showed a competition for the adsorption sites with the organic matter. They concluded that virus removal by activated carbon from a treated wastewater was not a dependable process. Gerba et al. (1975) found that virus (type 1 Poliovirus) removal from wastewater effluent by activated carbon was greatly improved by lowering pH to 3.5 to 4.5 or by reducing the concentration of organics by lime coagulation. Hydraulic loading in

reduced potential for inactivating viruses. Complete inactivation of Poliovirus 1 in secondary effluent after a 30 min contact period has been reported with free chlorine residuals of 0.2-0.4 mg/l (Burens and Sproul, 1967). A combined chlorine residual of 40 mg/l produced a 99.9 percent inactivation of Poliovirus 1 in settled wastewater with 30 min contact time. Only 50 percent or less of Poliovirus 1 was inactivated with a combined chlorine residual of 1 mg/l and a contact time of 30 min. Shuval (1969) reported 99.9 percent inactivation of Poliovirus 1 in wastewater effluent with 40 mg/l of applied chlorine and 10 min contact

Regarding the use of ozone in wastewater disinfection, Nebel et al. (1973) showed that 15 mg/l of ozone dose and a contact time of 5 min could bring about 100 percent virus inactivation in an activated sludge effluent. Burleson et al. (1975) observed rapid inactivation of several viruses by ozone in phosphate-buffered saline, but the viruses persisted significantly longer in secondary effluent.

3. SCOPE OF THE STUDY

Available literature indicates that all water and wastewater treatment procedures inactivate or remove viruses to some degree. It is also evident that virus removal efficiency of conventional wastewater treatment processes are rather erratic and cannot be relied upon because various factors such as sudden change in waste composition, large variation in flow, etc. significantly affect virus removal. As reuse practice increases, more stringent requirements will be placed on treatment of water and wastewater and more effective removal of viruses will be required. This necessitates a great need for investigating new methods of wastewater treatment which can ensure high virus removal and greater degree of dependability. Physicochemical treatment is one such approach whose advantages have been well demonstrated by Weber et al. (1970) in regard to removal of organics and nutrients. However, very few studies have been reported regarding virus removal efficiency of the physicochemical treatment processes and were concentrated on any one of the single unit processes thus being unable to present a comprehensive picture. A study of Sobsey et al. (1973) indicated 99.95 percent removal virus concentration in a prototype of a packaged sanitary wastewater treatment system employing physicochemical processes (comminution, chlorination, activated carbon adsorption, alum flocculation, and vacuum filtration using diatomaceous earth as a filter aid.

The present study was initiated to investigate the virus removal potential of various physicochemical processes, both separately and in combination, in the treatment of wastewater. Potential of bituminous coal in the physicochemical treatment of wastewater in terms of virus removal was also evaluated. Using virus seeded wastewater, study was undertaken along the following lines:

1. virus removal during plain sedimentation of raw wastewater,
2. removal of viruses during coagulation of raw wastewater using alum and ferric chloride,
3. virus sorption potential of bituminous coal and sand from raw and alum coagulated wastewater,
4. performance of bituminous coal, bituminous coal-sand and activated carbon as filter media for removing viruses during direct filtration of raw settled wastewater, and
5. removal of viruses as well as organics (C.O.D.), phosphate and turbidity during filtration of alum coagulated wastewater through dual-media bituminous coal-sand filter.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Virus

Bacterial virus MS2 (MS2 phage) against Escherichia coli A-19 was selected as the model virus for this study. This virus resembles human enteroviruses in size, shape, and type of nucleic acid. MS2 phage is stable, economical to culture and easily enumerated. Another important point in selecting this virus was the availability of considerable amount of data regarding its behaviour as a model virus in water and wastewater treatment studies.

MS2 phage has a single stranded RNA core surrounded by a lipid-free protein coat (isoelectric point, 3.9). It is polyhedron in structure having a diameter of 25 nm, and molecular weight of 3.7×10^6 (Overby et al., 1966) MS2 phage is similar to poliovirus in coat protein composition except that the amino acid residue histidine is absent (Levintow and Darnell, 1960; and Fraenkel-Conrat, 1968).

Bacteriophages behave in the same way as enteroviruses in most of the cases (Chang et al., 1958; and Gilcreas and Kelly, 1955). It was believed that the difference between naturally occurring viruses of concern, i.e., enteroviruses and those between the wild and attenuated laboratory strains of such viruses would offset the difference in behaviour between those viruses and MS2 phage. A study by Metropolitan Water Board, London (1971-73) on virus removal

by slow sand filtration showed practically identical results with poliovirus and MS2 phage. A look at Table 7 also shows slight differences in results obtained using animal and bacterial viruses.

4.1.2 Biological Media

L Broth

(Constituents per liter of water)

Bacto-Tryptone (Difco)	10.0 gm
Yeast Extract (Difco)	5.0 gm
NaCl	10.0 gm
Glucose	1.0 gm
2 M $CaCl_2$	1.0 ml

Adjusted to pH 7.0 with 1 N NaOH

L Agar

L Broth plus 15 to 20 gm/l Bacto Agar (Difco)

Soft Agar

L Broth plus 10 gm/l Bacto Agar (Difco)

4.1.3 Glassware

All glassware were soaked overnight in 0.3 percent D-300 Teelpol, Surfactants Private Limited, Bombay to minimize virus sorption onto glassware, followed by washing in tap water and final rinsing with distilled water. Sterilization was accomplished in a hot-air oven at 180°C for 2 hr or longer.

4.1.4 Filter Media

4.1.4.1 Sand

The sand used was silica sand obtained from the Kanpur Water Works. It was washed several times with tap water dried at 103°C for 24 hr, sieved to a geometric mean size (G_m) of 0.5 mm. Sphericity of this sand was 0.8 and porosity in the filter column was 0.35.

4.1.4.2 Coal

The coal used was high-grade Giridih bituminous coal obtained through the National Environmental Engineering Research Institute, Nagpur. This was selected because of its excellent virus sorption capacity among several Indian bituminous coals tested (Oza, 1974) and superior physical properties for use as a filter media (Sriramulu, 1975). The coal was crushed and sieved to a geometric mean size (G_m) of 1.0 mm, washed several times in tap water, dried at 103°C for 24 hr. Sphericity and porosity in filter column were 0.74 to 0.76 and 0.5, respectively.

4.1.4.3 Activated Carbon

Activated carbon used was Filtrasorb 400 (Calgon Corporation, Pittsburgh,(U.S.A.). The activated carbon was sieved to a geometric mean size (G_m) of 1.0 mm before using.

4.1.5 Chemicals

Chemicals used in coagulation experiments were Alum ($Al_2(SO_4)_3.16H_2O$) marketed by Sarabhai M. Chemicals Ltd., Baroda, and Ferric Chloride anhydrous ($FeCl_3$) marketed by Thomas Baker &

Co., Bombay. Other chemicals used were sodium chloride marketed by Sarabhai M. Chemicals Ltd., Baroda and calcium chloride ($CaCl_2.2H_2O$, marketed by Riedel-De Haen AG, Sleeze, Hannover, Germany. All these chemicals were of analytical reagent grade.

4.1.6 Wastewater

Indian Institute of Technology, Kanpur, campus raw domestic wastewater was used in all the experiments. Typical analysis of the wastewater is shown in Table 10. All analyses were performed according to the Standard Methods (1971).

4.2 Methods

4.2.1 Preparation and Enumeration of MS2 Phage

The initial stock cultures of MS2 phage and its host Escherichia coli A-19, were obtained originally from the Environmental Engineering Laboratory, University of Illinois at Urbana-Champaign and subsequent suspensions were prepared according to the following procedure.

A liter flask containing 900 ml of L Broth and equipped with an aeration device was inoculated with overnight grown Escherichia coli A-19 so as to get an A_{660} of 0.06-0.10. The flask was maintained at 37°C and aerated until the cell culture reached an A_{660} of 0.15-0.20. This corresponded to an early log growth phase of Escherichia coli A-19 (5×10^7/ml). The required amount of MS2 phage stock was then added at a multiplicity of 6-10. The aeration of the flask at 37°C was continued with A_{660} measurements at

TABLE 10

Characteristics of Raw Wastewater

pH	8.0
Turbidity, NTU	45.0
Conductivity, mhos/cm	1.5×10^{-3}
C.O.D. mg/l	120.0
C.O.D. to B.O.D. ratio	1.25
Ammonia Nitrogen as N, mg/l	17.60
Total Nitrogen as N, mg/l	24.30
Total phosphate as P, mg/l	8.9
Total Alkalinity as $CaCo_3$, mg/l	430.0
Total Coliforms no./ml.	20.4×10^3

intervals. First the absorbance would increase and later it might level off or gradually decrease indicating lysis. Five ml of chloroform was added after lysis had started and the lysate was stirred at high speed for 1 min using a magnetic stirrer. It was then kept at 4°C and stirred every 5 min. After 30 min, the lysate was centrifuged at low speed (5,900 x g for 10 min) to remove bacterial cell debris. The virus concentration of the clear supernatant was enumerated and stored at 4°C for subsequent seeding of wastewater as needed.

Soft agar technique of Adams (1959) as followed by Chaudhuri (1969) was adopted for enumeration of MS2 phage. Before assaying, the sample was diluted in L Broth to yield 100-300 plaques per

plate. A liquid top-agar mixture consisting of about 3 ml of soft agar at 45°C, 0.3 ml of a log growth phase culture of Escherichia coli A-19 cells, and 0.1 ml of the diluted virus sample was plated on a solidified bottom agar (L Agar) plates and incubated at 37°C for 6-8 hr. Plaques were counted with the aid of a Quebeck colony counter and reported as Plaque Forming Units per ml (PFU/ml). Triplicate plates were prepared from each sample to increase accuracy.

4.2.2 Sedimentation Experiments

Raw wastewater, seeded with MS2 phage so as to give a concentration of about 1 x 10^5 PFU/ml, was filled to the top of a liter graduated cylinder, thoroughly agitated and was allowed to settle. Samples were taken from the top surface at predetermined time intervals and virus concentration enumerated.

Data on virus removal during 2 hr settling of raw wastewater were also collected before conducting coagulation experiments since a sedimentation time of 2 hr was employed before coagulation. Six liters of seeded raw wastewater with an input virus concentration of about 1 x 10^5 PFU/ml was settled in a plastic container. Initial and final sample at the end of a 2 hr settling period were plated for virus enumeration.

4.2.3 Coagulation Experiments

All coagulation experiments were simple, batch type jar tests conducted using a six place multiple stirring apparatus (Phipps and Bird, Richmond, Va.). In the procedure, raw wastewater

seeded with MS2 phage to a concentration of 1 x 10^5 PFU/ml was first settled in a plastic container for 2 hr. In six one liter beakers 750 ml of this settled wastewater were placed and coagulated with alum for ferric chloride using flash mix @ 100 rpm for 1 min followed by slow mix @ 30 rpm for 20 min and 30 min of quiescent settling. The first beaker always served as a control to which no coagulant was added. At the end of the settling period, supernatant from all the beakers were analysed for pH, turbidity and virus concentration. For experiments to determine the optimum pH for coagulation, 1 N NaOH or 1 N HCl was used for pH adjustment.

4.2.4 Batch Sorption Experiments

To study the potential of sand and coal as sorbents in the removal of viruses from raw settled and alum coagulated wastewater, batch sorption tests were carried out in non-flow agitated system using a rotating shaker. The reaction mixture was either virus seeded raw settled wastewater or seeded wastewater coagulated with optimum alum dose. For each set, three 300 ml BOD bottles containing 295 ml of the reaction mixture were employed. One bottle served as control (no sorbent) and the other two bottles served as reactors with either 10 gm/l of coal or sand as sorbents. Samples were withdrawn from the bottles at predetermined time intervals for virus concentration. All experiments were performed at room temperature (30 to 32°C).

4.2.5 Filtration Experiments

A laboratory bench-scale filtration apparatus was employed in this study. It consisted of a glass filter column (pyrex glass), 2.54 cm (1 in.) ID and 1 m long, with suitable ports for virus and turbidity sampling, and headloss measurements. A 60 l plastic container served as the overhead storage tank and provided a filtration head of about 200 cm. Constant filtration rates were maintained by manually adjusting the filter outlet. Plastic manometers, 0.4 cm OD, were used for headloss measurements.

A set procedure was followed for the filtration experiments. Prior to initiating an experiment (filter run), a 0.3 percent B-300 Teepol solution was introduced into the filtration system and retained in the apparatus for 2 hr. Then the solution was removed and the system washed with water. The filter column was then filled with water and the required amount of filter media (coal or activated carbon) for a desired depth of filter bed placed in the column. In case of dual-media filter, first 9 in. of sand bed was placed in the filter column followed by a 9 in. of coal bed above the sand. Influent wastewater was then placed in the overhead tank and the experiment initiated at a desired filtration rate by adjusting the filter outlet. Headloss measurements were taken at various depths and time intervals during the run. Samples for virus enumeration and turbidity were taken at desired depths and at the filter outlet while influent samples were collected from a port located immediately above the filter bed. Samples for virus enumeration were immediately diluted in L Broth, stored at 4°C and

plated as soon as possible but always within 2 hr of collection. Influent and effluent samples were also occasionally checked for pH to record any change in pH during a run. Filter runs were usually of 6 hr duration or less except for one experiments which was continued for 18 hr to simulate plant conditions. Phosphate and C O.D. were also monitored during long run.

Filtration runs were performed with the virus seeded wastewater and preceded by plain sedimentation (2 hr) or plain sedimentation followed by alum coagulation and subsequent settling. For filter runs with alum coagulated and settled wastewater, 50 liters of raw wastewater seeded with desired concentration of the virus was settled for 2 hr and coagulated (1 min flash mix and 20 min slow mix using a shaft stirrer followed by 30 min of settling) with the optimum alum dose. The supernatant was then transferred to the storage tank. Samples for turbidity and virus enumeration were taken before and after coagulation.

5. RESULTS AND DISCUSSION

All experimental results are presented in either graphical or tabular form. For the most part, only typical results are presented. In order to fecilitate the presentation, a discussion of the results follows each phase of the experimental work.

5.1 Virus Removal During Plain Sedimentation

Table 11 shows virus and turbidity removals at various times during plain sedimentation of MS2 phage seeded raw wastewater whereas Table 12 shows removals after 2 hr of plain sedimentation. It is seen that plain sedimentation removes about 23-29 percent of the seeded viruses and the removal is not very consistent as reported in the literature (Engelbrecht, 1976). However, a two hour plain sedimentation normally employed in wastewater treatment may remove about 21-28 percent of the viruses present. It may be mentioned here that both the nature and amount of turbidity as well as virus concentration may influence virus-solids association thereby affecting their removal by sedimentation. However, the effect of these two parameters was not evaluated in the present study.

5.2 Virus Removal During Coagulation

Figure 1 shows removal of MS2 phage and turbidity from wastewater during alum and ferric chloride coagulation. Original virus concentration, pH and turbidity values reported in the figure were

TABLE 11

Virus Removal During Plain Sedimentation

Time (hr)	Turbidity (NTU)	Virus Concentration x 10^{-4} (PFU/ml)	Virus Removal (percent)
0	44.0	13.6	
0.5	46.0	10.8	23.00
1.0	42.0	10.0	26.50
2.0	38.0	10.4	23.60
3.0	36.0	9.8	27.95
4.0	34.0	9.6	29.40

Temperature : 25°C; pH 7.8

TABLE 12

Virus Removal During Two Hour Sedimentation Before Coagulation Experiments

Sl. No.	Turbidity (NTU)	pH	Virus Concentration x 10^{-4} (PFU/ml)		Virus Removal (percent)
			Influent	Effluent	
1	34.0	8.0	2.90	2.10	27.58
2	50.0	8.0	11.40	8.85	22.36
3	37.0	7.9	8.60	6.80	20.93
4	31.0	7.8	14.00	10.30	26.42

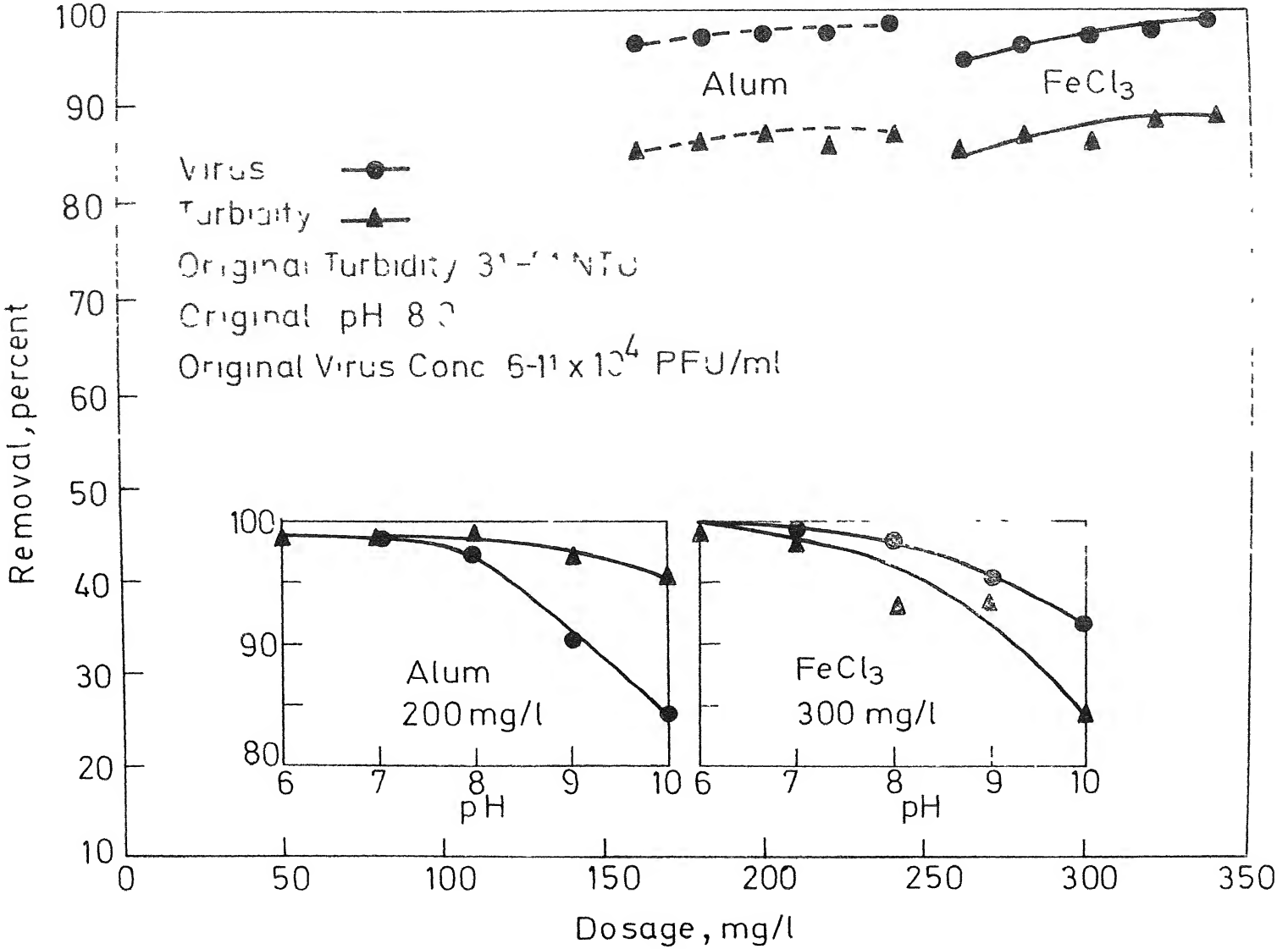

Fig 1 Removal of MS2 Phage from Seeded Wastewater During Alum and Ferric Chloride Coagulation

the average values of these parameters before plain sedimentation preceding the coagulation experiments. For both alum and ferric chloride, background exponents were conducted to obtain an initial optimum coagulant dosage and the final experiments were performed using close range dosages in the vicinity of the initial optimum dose. It is seen that for both the systems turbidity removal paralleled virus removal. The maximum virus removal observed was identical (99.1 percent) for both the coagulants; however, the dosages were 240 and 340 mg/l for alum and ferric chloride, respectively. The pH range for best performance of both the systems was observed to be between 6 and 7. These experiments were conducted with alum and ferric chloride dosages of 200 and 300 mg/l. respectively which were the observed optimum in the background experiments.

The above results are comparable to those reported by Shelton and Drewry (1973) for removal of f2 phage from domestic wastewater using alum and ferric chloride. However, the coagulant dosages observed in the present study are significantly higher than those reported by them (76 mg/l of alum and 109 mg/l of ferric chloride). This discrepancy is apparently due to the variation in the wastewater characteristics.

It may be concluded from the limited data available from the present study that alum is a more effective coagulant for wastewate than ferric chloride in terms of virus removal. Moreover, effluents were found to be coloured when ferric chloride was used as coagulant which is a disadvantage. It was also concluded by

Shelton and Drewry (1973) that alum was the best primary coagulant when all parameters (dose, percent removal of viruses, turbidity and C.O.D., and colour) were considered. In all subsequent studies alum dosage of 240 mg/l was used whenever coagulation was performed

5.3 Virus Sorption Potential of Giridih Coal and Sand

Batch sorption tests were conducted to investigate the virus sorption potential of Giridih coal and sand from raw as well as alum coagulated wastewater (Fig. 2). The nature of the kinetics of MS2 phage sorption observed is similar to the findings of Sriramulu (1975). For both raw and coagulated wastewater coal demonstrated better sorption potential. Increased virus sorption from the coagulated wastewater may be attributed to the reduction of organics during coagulation which interfere with virus sorption as demonstrated by Oza (1974).

5.4 Direct Filtration of Raw Settled Wastewater

The objective of this phase of the study was to investigate the relative performance of Giridih coal, activated carbon and Giridih coal-sand dual-media filters in the direct filtration of raw settled wastewater.

5.4.1 Single Medium Filters

Figure 3 shows the performance of two different depths of Giridih coal (45.70 and 76.20 cm) and activated carbon (48.26 and 71.12 cm) at a filtration rate of 4.9 m/hr, corresponding to a

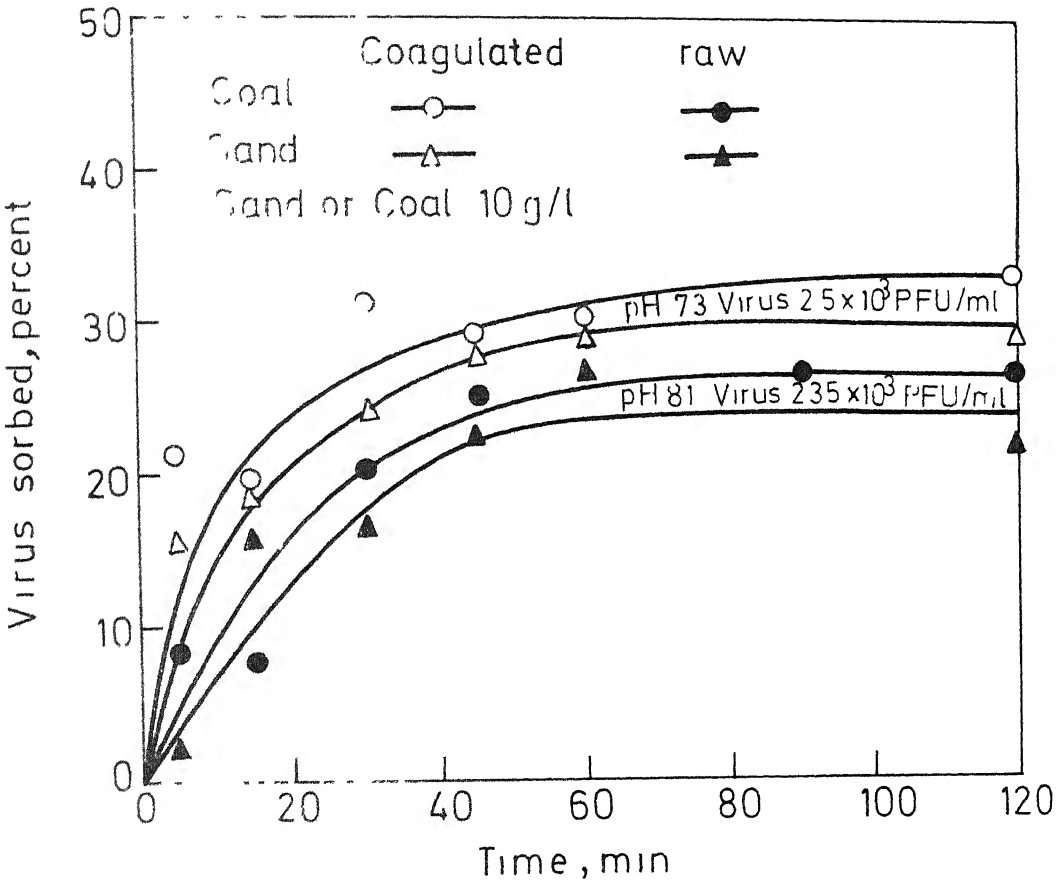

Fig 2 Kinetics of Sorption of MS2 Phage From Wastewater on Giridih Coal and Sand

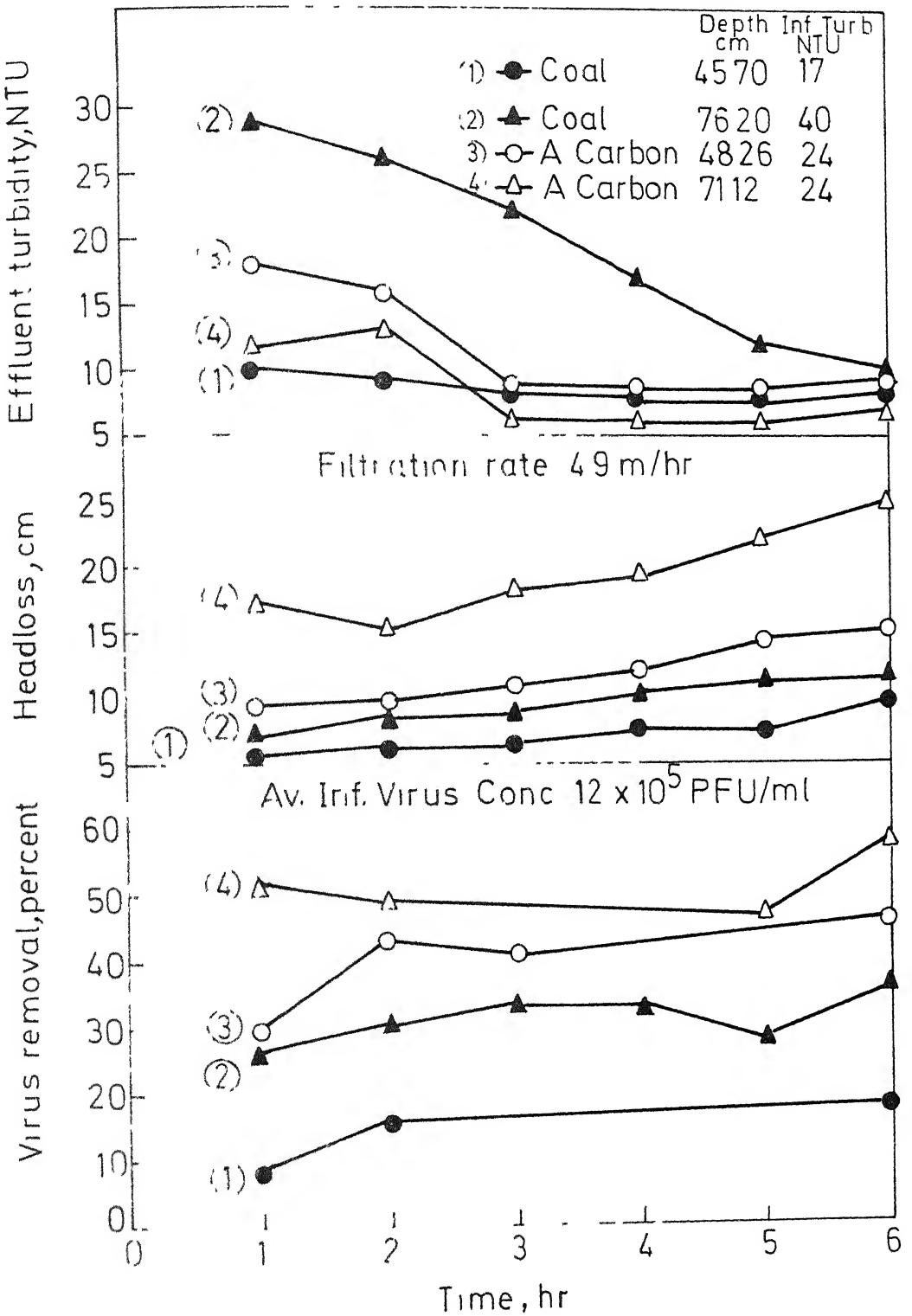

Fig 3 Performance of Giridih Coal and Activated Carbon During Direct Filtration of Raw Settled Wastewater

loading rate of 81.65 lpm/sq m. The average concentration of virus and turbidity reported are that of influent to the filter i.e. after plain sedimentation of the wastewater. At higher depths viru removal efficiency of activated carbon (59 percent) was found to be better than that of Giridih coal (36.2 percent) at the same filtration rate. The virus removal efficiency of both Giridih coal and activated carbon increased as the depth increased at the same filtration rate. It is seen from the graph that high turbidity removals are observed at greater depths of both activated carbon and Giridih coal filters corresponding to higher removal of viruses Hence it may be inferred that the viruses possibly attach to the solids in wastewater. The subsequent removal of solids during filtration may be responsible for the removal of viruses. However, Gerba et al (1975) reported column length did not affect overall percent virus removal. This appears due to the change in characteristics of filter influent. They used chlorinated secondary effluen from a trickling filter, which was further passed textile filters (smallest porosity 1 μm) as influent, whereas raw settled wastewat was used in the present study.

Both Gerba et al. (1975) and Sproul et al. (1971) have report higher virus removal during slower filtration rates. Gerba et al. (1975) reported average virus removal of 26 percent at 81.65 lpm/ sq m whereas in the present study average virus removal of about 50 percent was observed at the same flow rate. Probable reason for increased virus removal might be the presence of solids.

5.4.2 Dual-Media Filter

The performance of Giridih coal-sand dual-media filter at a filtration rate of 9.8 m/hr (163.30 lpm/sq m) is presented in Fig. 4. The average concentration of virus and turbidity reported are that of influent to the filter. The virus removal efficiency of Giridih coal-sand dual-media filter appears to be very good (92 percent) when compared to Giridih coal and activated carbon filters reported previously. However, the turbidity removal was comparatively less in the Giridih coal-sand filter. Working on filtration of water Sriramulu (1975) reported that the virus removal efficiency of Giridih coal-sand dual-media filter improves in the presence of turbidity. It was further reported that there was concurrent removal of both turbidity and virus. In the present investigation using dual media filter while significant removal of virus was obtained in spite of poor turbidity removal. However, in the previous section the removal efficiencies of both virus and turbidity was quite high in single medium filtration. To explain this disparity further probing may be required.

5.5 Filtration of Alum Coagulated Wastewater

Experiments conducted in this phase were to study the virus removal efficiency of Giridih coal-sand dual-media filter, under different rates of filtration. Also, overall removal of viruses as well as organics (C.O.D.), phosphate and turbidity during long filtration run, was studied. Figure 5 shows the performance of Giridih coal-sand dual-media filter and various filtration rates

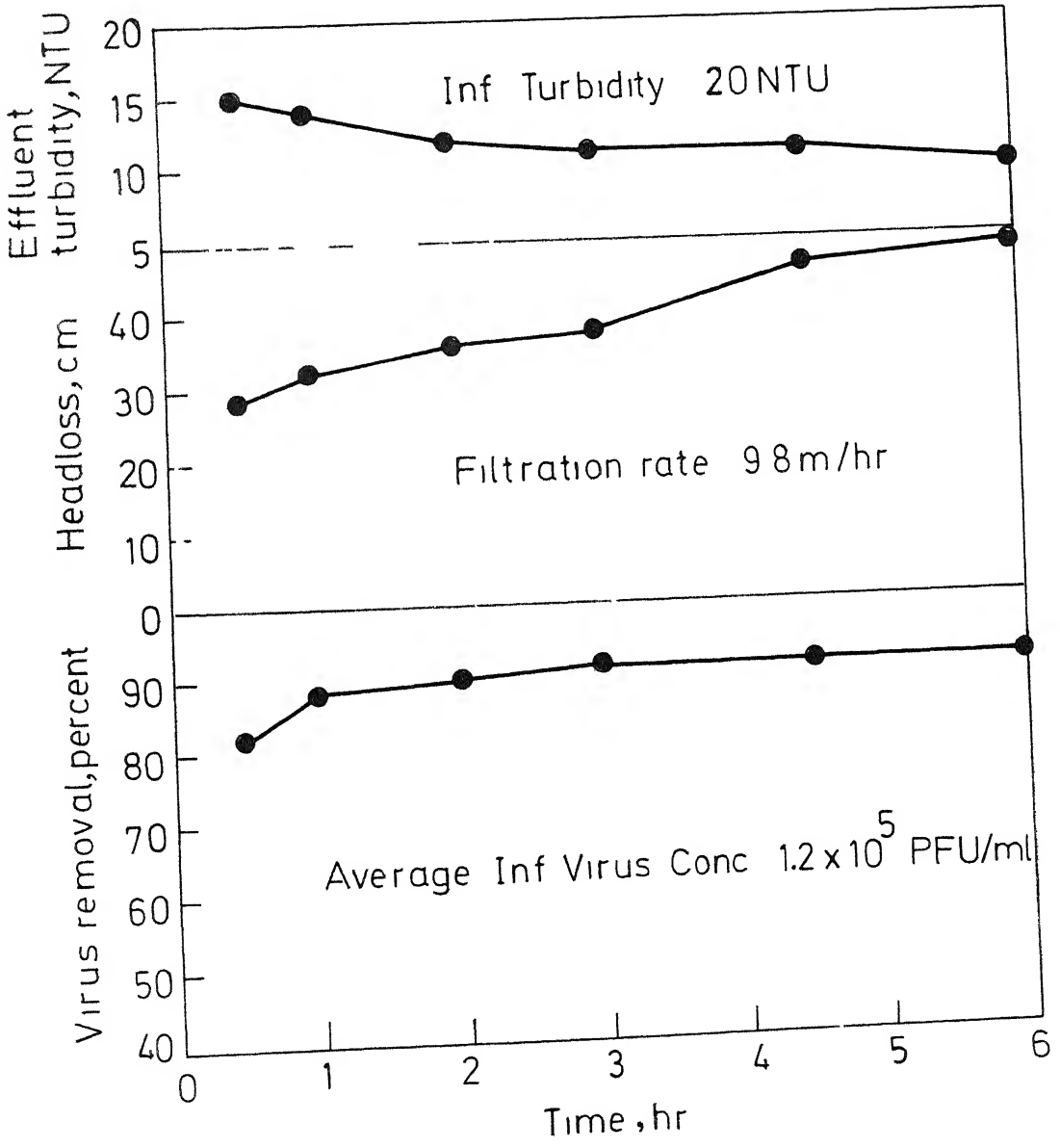

Fig 4 Performance of Giridih Coal-Sand Dual-Media Filter During Direct Filtration of Raw Settled Waste-water

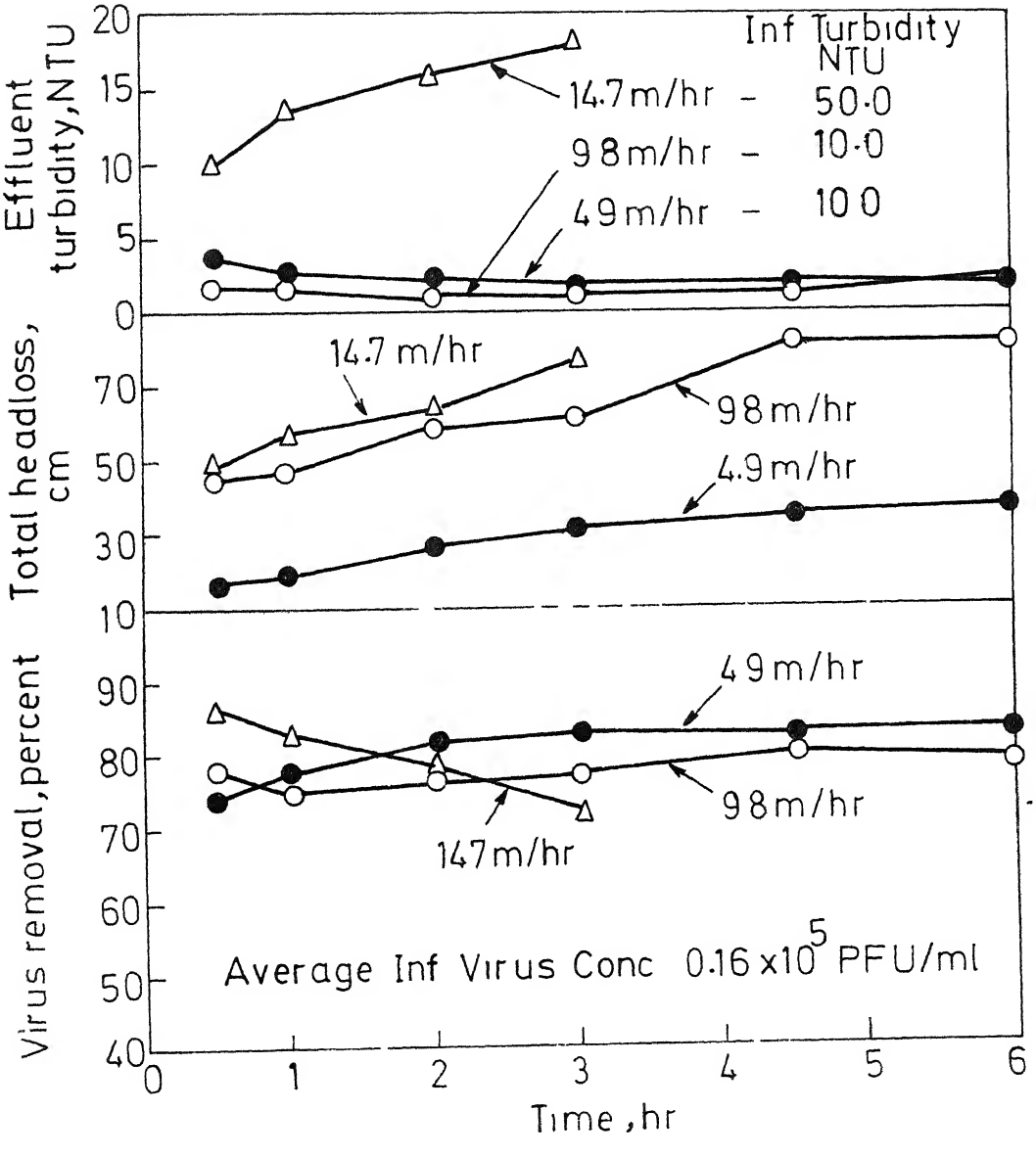

Fig 5. Performance of Giridih Coal-Sand Dual-Media During Filtration of Alum Coagulated Wastewater at Various Filtration Rates

(4.9, 9.8, and 14.7 m/hr). All the values reported in Fig. 5 are that of influent to the filter and the virus removal efficiency corresponds to filter efficiency.

It is seen from Fig. 5 that performance of Giridih coal-sand dual-media filter was better at slower rate of filtration (4.9 m/hr). However, virus removal efficiency at filtration rates of 4.9 m/hr and 9.8 m/hr paralleled and are comparable (83.5 and 81.2 percent). Turbidity removals is also high and are comparable at the above filtration rates. The virus removal efficiency, at the filtration rate of 14.7 m/hr, was maximum (86.4 percent) initially (0.5 hr) and rapidly decreased as filtration progressed (72.15 percent at the end of 3 hr). It was also observed that effluent turbidity rapidly increased with filtration run. Hence the longer filtration run was conducted at 9.8 m/hr.

The performance of the Giridih coal-sand dual-media filter regarding virus, turbidity, phosphates, and C.O.D. removal during longer filtration run (18 hr) at a filtration rate of 9.8 m/hr is shown in Fig. 6. All the values reported in Fig. 6 corresponds to seeded raw wastewater and hence the removal efficiency corresponds to efficiency of the complete system viz. plain sedimentation (2 hr), alum coagulation and filtration through Giridih coal-sand dual-media.

It is evident from Fig. 6 that a consistent and good removal of viruses can be anticipated during plain sedimentation, alum coagulation and filtration through Giridih coal-sand dual-media. However, the overall virus removal efficiency (98 percent) reported

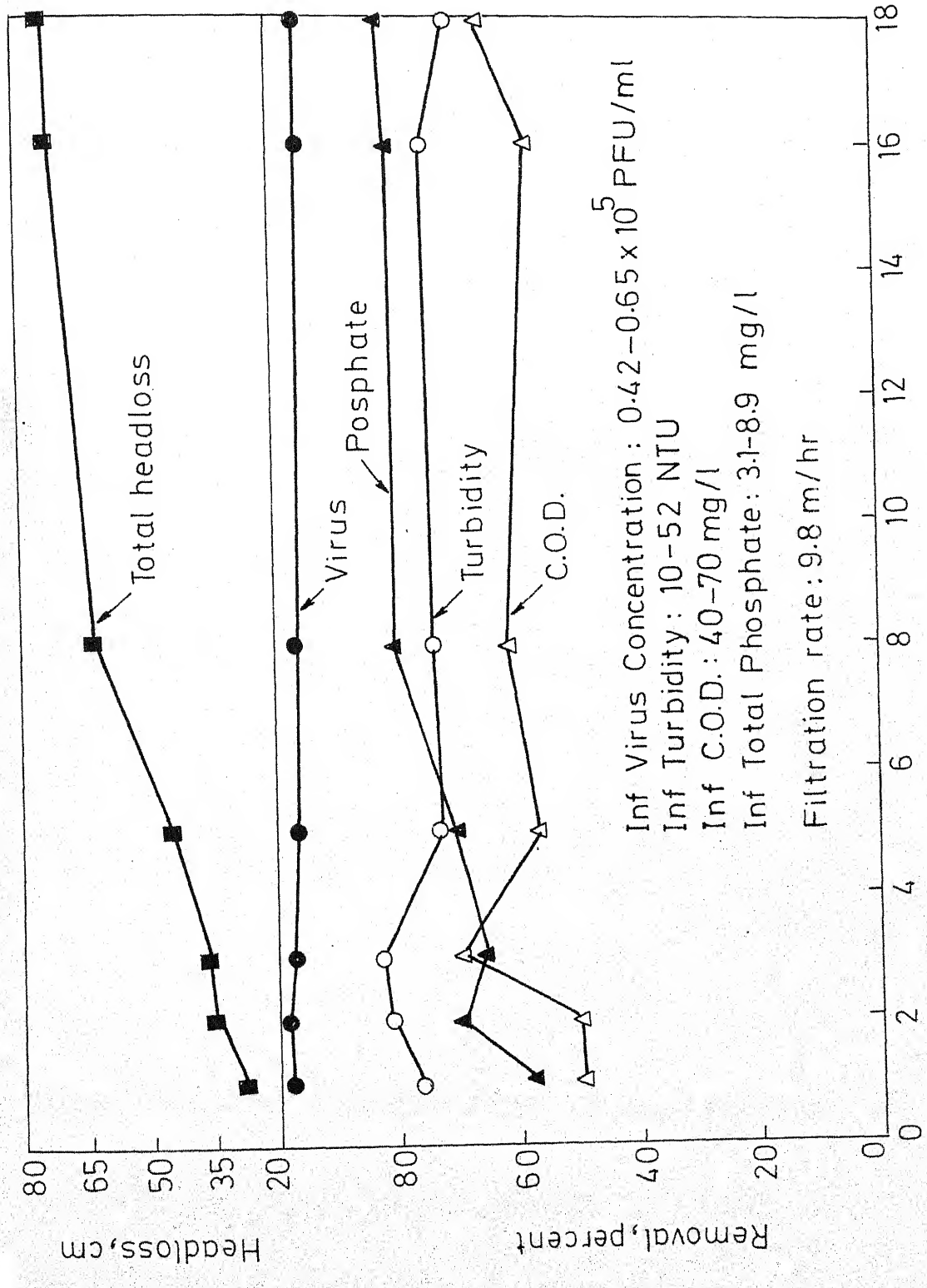

Fig.6 Removal Virus, Phosphate, Turbidity and C.O.D. During Filtration of Alum Coagulated Wastewater Through Giridih Coal-Sand Dual-Media Filter

is less than expected because of the low efficiency of coagulation system (83.25 percent) performed prior to filtration which does not corresponds to the efficiency of jar test. Phosphate, C.O.D., and turbidity removal efficiency varied slightly with time and the values are comparable to those reported by Weber et al (1971).

6. SUMMARY AND CONCLUSIONS

The results of the present study show that the physicochemical treatment processes are effective in removal of viruses from MS2 phage seeded wastewater. Plain sedimentation of raw wastewater for 2 hr showed an average virus removal of 21-28 percent. Both alum and ferric chloride coagulation were effective in removing viruses as well as turbidity. Maximum virus removal observed for alum and ferric chloride coagulation was 99.1 percent at dosages of 240 mg/l and 340 mg/l, respectively. Giridih coal sorbed MS2 phage better from alum coagulated and raw wastewater, than sand. Activated carbon filter bed (71.12 cm depth) removed more viruses (59 percent, compared to Giridih coal bed (76.20 cm depth, 36.2 percent virus removal) when raw settled wastewater was filtered at a rate of 4.9 m/hr. Higher virus removal (92 percent) during filtration of raw settled wastewater through Giridih coal-sand dual-media could not be accounted and further work is necessary for its evaluation. Performance of Giridih coal-sand dual-media when alum coagulated wastewater was filtered at rates of 4.9 m/hr and 9.8 m/hr was commendable. A consistent and good removal of viruses may be expected in system consisting plain sedimentation, alum coagulation and filtration through Giridih coal-sand dual-media.

Based on the findings of this investigation using a MS2 phage seeded wastewater, the following conclusions may be drawn:

1. Physicochemical processes comprising plain sedimentation, alum coagulation and Giridih coal-sand dual media filter may emerge as a replacement to biological processes.
2. Plain sedimentation may remove viruses to an extent of 21-28 percent.
3. Both alum and ferric chloride coagulation are effective in removing viruses (99.1 percent). But, alum was found to be a better coagulant.
4. Direct filtration of raw settled wastewater through Giridih coal or activated carbon are not effective.
5. Filtration of alum coagulated wastewater through Giridih coal-sand dual-media filter at filtration rates of 4.9 and 9.8 m/hr is effective.

7. ENGINEERING SIGNIFICANCE AND SUGGESTIONS FOR FURTHER WORK

7.1 Engineering Significance

The present study is significant from the viewpoint of a systematic study of physicochemical processes for virus removal potential. Physicochemical treatment of wastewater is fast replacing biological processes and is being studied thoroughly both at laboratory and field levels. A developing country like India, where most of domestic wastewater in untreated, may choose physicochemical treatment processes instead of biological processes. Furthermore, it is felt that this study will provide enough impetus and background information for future laboratory as well field studies in this area.

7.2 Suggestions for Further Work

Based on the results of this study it is felt that further work should be pursued in the following areas:

1) A detailed study should be undertaken to evaluate the mechanisms of virus removal and the role of other parameters like, organics, nutrients etc. in coagulation and filtration processes. This is expected to help in developing the design standards for virus removal in physicochemical treatment processes.

2) Study should be conducted to probe the anomaly found in direct filtration of raw settled wastewater through Giridih coal-sand dual-media filter which could not be accounted for in this investigation.

3) Studies should be conducted on a pilot plant scale to evaluate the virus removal efficiency of physicochemical treatment processes of wastewater.

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